Protective activity of recombinant cytokines against Sendai virus and herpes simplex virus (HSV) infections in mice

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The efficacy of recombinant cytokines such as murine interferon-γ (IFN-γ), human granulocyte colony-stimulating factor (G-CSF), mouse granulocytic-macrophage colony-stimulating factor (GM-CSF) and human interleukin-1β (IL-1β) has been examined for augmentation of host resistance against Sendai virus and herpes simplex virus (HSV) infections. All four cytokines were found to protect mice against Sendai virus infection. IFN-γ afforded protection when administered intranasally but not intravenously several days before the infection. Intranasal administration of G-CSF one day before the infection was the most effective administration route and timing. Intranasal administration of GM-CSF was found to afford protection 1 or 3 days before the infection. IL-1β demonstrated therapeutic activity against Sendai virus infection after intranasal administration on the same day as the infection. When each of the cytokines was administered subcutaneously four times daily into cyclophosphamide-treated mice before intravenous infection with HSV, only GM-CSF revealed any protective activity.

Keywords: Sendai virus; cytokines; non-specific host resistance; infection; herpes simplex virus

Introduction

It has been found that host resistance against Sendai virus and herpes simplex virus (HSV) infections can be enhanced by the administration of such bacterial immunoadjuvants as whole mycobacteria, lipopolysaccharides (LPS) derived from Gram-negative bacteria, muramyl dipeptide (MDP), which is the minimal unit to show immunoadjuvanticity[1–4]. Recently, host resistance to the infections has been shown to be augmented by muramyl tripeptide-phosphatidylethanolamine (MTP-PE), which is a lipophilic derivative of MDP[5]. We have previously reported that N^{α}-acetylmuramyl-L-alanyl-D-isoglutaminyl-N^{ε}-stearoyl-L-lysine [MDP-Lys(L18)] can protect mice against Sendai virus infection by intranasal administration and that macrophages activated by MDP-Lys(L18) are able to suppress the growth of Sendai virus in the lungs of normal mice[6,7] and can augment host resistance against HSV infection in mice treated with cyclophosphamide (Cy)[7a].

MDP-Lys(L18) is a potent inducer of interleukin-1 (IL-1) among macrophages *in vivo* and colony-stimulating factor (CSF) *in vivo*[8]. Infections of *Listeria monocytogenes*, *Salmonella typhimurium* and ectromelia virus, resistance to which largely depends upon macrophages, have caused the induction of CSF in sera[9–11]. Alveolar macrophages exposed to influenza virus have produced IL-1 *in vitro*[12]. These results suggest that similar factors would be likely to have a role in natural host resistance to microbial infections. Recently, recombinant cytokines such as IL-1, granulocyte CSF (G-CSF), granulocyte-macrophage CSF (GM-CSF) as well as interferon-γ (IFN-γ) have been found to protect mice against *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, *Candida albicans* infections in normal or neutropenic mice[13–15].

In this study, we attempted to evaluate the anti-infectious activity of G-CSF, GM-CSF, IL-1 and IFN-γ against Sendai virus infection as well as against HSV infection in Cy-treated mice.

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Materials and methods

Mice

Specific pathogen-free, male inbred Balb/c slc mice were obtained from the Shizuoka Experimental Animal Center and maintained in the Laboratory of Animal Experiment, Institute of Immunological Science, Hokkaido University, under laminar-flow conditions. All mice were used at the age of 4–5 weeks. Water and a pelleted diet (Nihon Nosan Kogyo Co. Ltd, Yokohama, Japan) were supplied *ad libitum*.

Reagent

Recombinant mouse interferon-γ (IFN-γ) (0.7 mg ml^{-1}, specific activity 10^7 U mg^{-1}), prepared by Schering-Plough Corporation, was generously donated by the Suntory Co. Ltd (Osaka, Japan). Recombinant human granulocyte colony-stimulating factor (G-CSF) (50 μg ml^{-1}, specific activity 3×10^7 U mg^{-1}) was kindly supplied by Chugai Pharmaceutical Co. Ltd (Tokyo, Japan). Recombinant human interleukin-1β (IL-1β) (0.1 mg ml^{-1}, specific activity 2×10^6 U mg^{-1}) was kindly supplied by Otsuka Pharmaceutical Co. Ltd (Tokushima, Japan). Recombinant granulocyte-macrophage colony-

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stimulating factor (GM-CSF) (1.17 mg ml^{-1}, specific activity 10^9 U mg^{-1}) was kindly provided by Sumitomo Pharmaceutical Co. Ltd (Osaka, Japan). Those recombinant cytokines were diluted with phosphate-buffered saline (PBS) containing 0.3% bovine serum albumin (BSA). In the preliminary experiment, we observed that the diluent did not have any effect on protection against Sendai virus or HSV infections. N^{α}-Acetylmuramyl-L-alanyl-D-isoglutaminyl-N^{ϵ}-stearoyl-L-lysine [MDP-Lys(L18)] was donated by Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan)

Protection against Sendai virus infection

Details of the methods have been reported previously[6]. The Sendai strain of parainfluenza type I virus was purchased from Flow Laboratories Inc., Rockville, MD. This virus was passaged for 11 generations in suckling C3H/He mice; after the 11th passage the lungs were homogenized in PBS and the supernatant fluid was dispersed in ampoules in 1 ml amounts, frozen and stored as the stock virus suspension at −70°C until use. For each experiment, an ampoule was thawed and 0.03 ml of the virus suspension was administered intranasally under light ketamine (Ketaral-50, Sankyo Co. Ltd, Tokyo, Japan) anaesthesia. When the infectious inoculum was assayed in LLC-MK2 cells (kindly donated by Dr H. Kida, Faculty of Veterinary Science, Hokkaido University), it was found to contain one $10^{4.4}$ haemoadsorption (HAD) unit per 0.03 ml. [illegible] were [illegible] up to 21 days after the infection. Probability values were calculated by applying the Mann–Whitney *U* probability test to the mean survival times of the treated group and that of the control group.

Protection against he[illegible] infection

Herpes simplex virus (HSV) type 1 strain MacIntyre ATCC VR535 was provided by Dr H. Sakaoka (School of Dentistry, Hokkaido University). A $10^{2.4}$ plaque-forming unit (p.f.u.) of HSV was injected intravenously in the mice which had received intraperitoneally cyclophosphamide (Sionogi Pharmaceutical Co. Ltd, Osaka, Japan) at a dose of 4 mg per mouse one day before the infection. Statistical analysis was performed as described above.

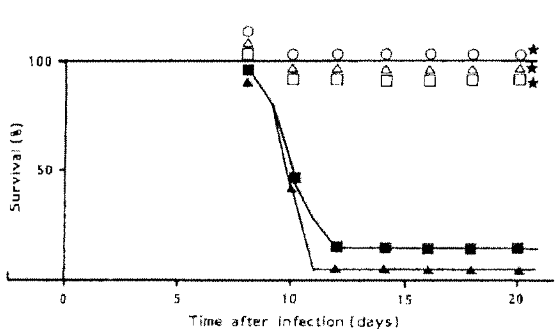


Figure 1 Protective activity of IFN-γ against Sendai virus infection in mice. Seven or 8 Balb/c mice were treated with various doses of IFN-γ 3 days before infection with Sendai virus ($10^{4.4}$ HAD per mouse). ○, 10^3 U (i.n.); △, 10^2 U (i.n.); □, 10 U (i.n.); ■, 10^3 U (i.v.); ▲, control; ★, $p < 0.001$

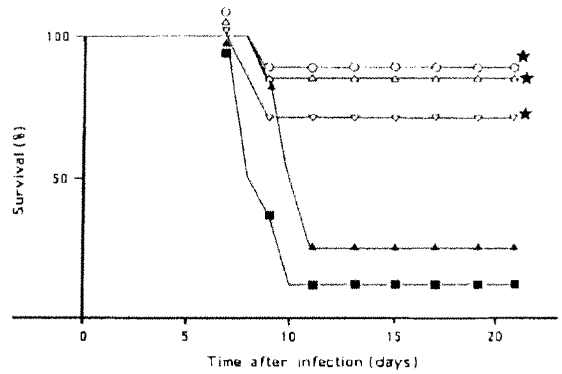


Figure 2 Effect of time intervals between treatment with IFN-γ and infection with Sendai virus. Seven Balb/c mice were given 100 U IFN-γ i.n. on various days before infection with Sendai virus ($10^{4.4}$ HAD per mouse). ○, 5 days before; △, 3 days before; ▽, 1 day before; ■, simultaneously and one day after infection; ▲, control; ★, $p < 0.001$

Results

Protective activity of IFN-γ on Sendai virus infection

The mice infected with Sendai virus died with severe pneumonitis 7–15 days after the infection (*Figure 1*). In the first experiment, we compared the protective activity of [illegible] administered intranasally (i.n.) or intravenously (i.v.). All the mice that had received 10, 10^2 and 10^3 U IFN-γ i.n. [illegible] days before infection survived for 21 days after infection, whereas [illegible] U [illegible]-γ i.v. was not effective (s[illegible]%). The survival rate of the control group was also 13%.

We next examined the effect of the timing of IFN-γ administration on its protective activity against Sendai virus infection (*Figure 2*). Although i.n. administration of 100 U IFN-γ either 5 days, 3 days or 1 day before the infection, showed potent protective activity (88%, 88% and 83%, respectively), postinfection administration (simultaneously with and 1 day after the infection) had no effect (13%). The survival rate of the control group was 25%.

Protective activity of G-CSF and GM-CSF on Sendai virus infection

When the mice were given 2.0 μg G-CSF one day before infection, the survival rate was significantly higher than that of the control group (75%, *Table 1*, Experiment 1). Treatment 3 days before infection showed a slight protective activity (43%) and all the mice (which were injected simultaneously and 1 day after the infection) died within 21 days of infection. Subcutaneous (s.c.) or i.v. administration of 2.0 μg G-CSF 1 day before infection was not effective for protection aginst Sendai virus infection (*Table 1*, Experiment 2). Although the data are not shown, 2.0 μg G-CSF was the minimal effective dose for affording resistance to infection in our experimental conditions. The survival rate of the mice that received 2.0 μg G-CSF s.c. or i.v. 1 day before infection was similar to that of the control group (14%, 14% and 0%, respectively) (*Table 1*, Experiment 2). Intranasal administration of GM-CSF augmented host resistance to infection either 1 or 3 days before infection (*Table 1*, Experiment 3).

Protective activity of rIL-1β on Sendai virus infection

The results presented above show that i.n. administration of IFN-γ and G-CSF before infection afforded a higher rate of protection against Sendai virus infection than i.v. or s.c. administration. Neither simultaneous administration nor postadministration of either cytokine was effective in increasing resistance to Sendai virus infection. As IL-1β has been shown to have some therapeutic effect in controlling microbial infection in mice[13,14], we examined its protective or therapeutic activity against Sendai virus infection by i.n. administration. Pretreatment with 0.2 μg IL-1β either 3 days or 1 day before infection afforded protection against infection (*Figure 3a, b*). Although the simultaneous administration of IL-1β (2 h after infection) was remarkably effective, the post-administration of 0.2 μg IL-1β either 1 day or 3 days after infection was not effective.

Table 1 Protective activity of G-CSF and GM-CSF against Sendai virus infection in mice

Experiment no.	Treatment	Schedule[a] (day)	Route	Survivors/total on day 21	p^b
1	G-CSF	−1	i.n.	6/8	$p<0.001$
		−3	i.n.	3/7	$0.02<p<0.05$
		0, +1	i.n.	0/7	
	Control			0/7	
2	G-CSF	−1	i.n.	3/7	$0.02<p<0.05$
		−1	s.c.	1/7	
		−1	i.v.	1/7	
	Control			0/7	
3	GM-CSF	−3	i.n.	6/6	$p<0.001$
		−1	i.n.	6/6	$p<0.001$
	Control			0/7	

[a]In each experiment, 2.0 μg G-CSF and GM-CSF were administered. Minus values indicate days before infection, plus values are days after infection. [b]Probability values were calculated by the Mann–Whitney *U* test

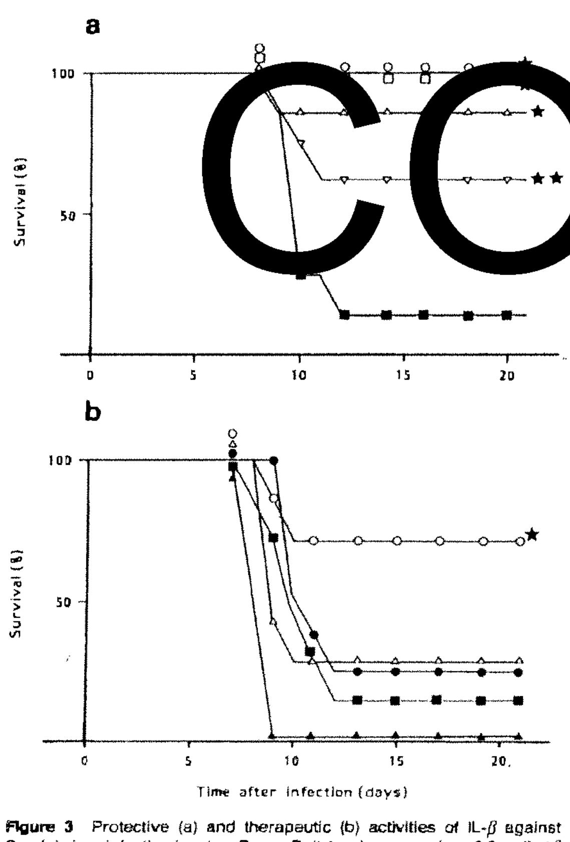
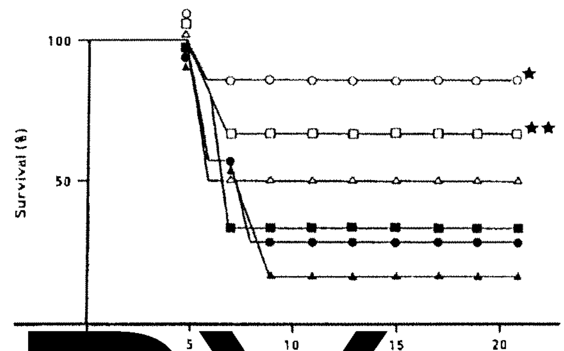


Figure 3 Protective (a) and therapeutic (b) activities of IL-β against Sendai virus infection in mice. Seven Balb/c mice were given 0.2 μg IL-1β i.n. on various days before or after infection with Sendai virus ($10^{4.4}$ HAD per mouse. (a) MDP-Lys(L18) (10 μg) was administered i.n. 1 day before infection. ○, 3 days before, □, 1 day before; △, simultaneously; ▽, MDP-Lys(L18); ■, control; ★, $p<0.001$; ★★, $0.02<p<0.05$. (b) ○, Simultaneously; △, 5 days after; ■, 3 days after; ▲, 1 day after infection; ●, control; ★, $0.02<p<0.05$

Table 2 Protective activity of G-CSF, and IFN-γ against HSV infection in mice which received cyclophosphamide (Experiment 1)

Sample	Treatment[a] On day	Treatment[a] Daily dose	No. of survivors/total on day 21	p value[b]
G-CSF	−4, −3, −2, −1	2.0 μg	1/7	
IFN-γ	−4, −3, −2, −1	100 U	3/7	
MDP-Lys(L18)	−3, −1	100 μg	5/7	<0.001
Control			2/7	

[a]Each of the samples was administered s.c. on the indicated days before infection. Cyclophosphamide was injected i.p. 1 day before infection at a dose of 4 mg. Mice were infected with HSV ($10^{2.4}$ p.f.u.) i.v. [b]Probability values were calculated by the Mann–Whitney *U* test



T[illegible]fter infe[illegible] (days)

Fi[illegible] 4 Protectiv[illegible]ivity of [illegible]β and [illegible]-CSF against HSV infection in mi[illegible] which receiv[illegible]yclophos[illegible]. Eight BALB/c mice were given IL-[illegible]bcutaneous[illegible]ays before infection with HSV. M[illegible] (100 μg per mouse) w[illegible]dministered s.c. both 3 days and 1 da[illegible]efore infection. Cyclophospha[illegible]e (4 mg per mouse) was injected int[illegible]ritoneally 1 day before infec[illegible]. ○, MDP-Lys(L18) (100 μg per m[illegible]e); □, GM-CSF (2 μg per [illegible]use); ■, GM-CSF (0.2 μg per mouse); △, IL-1β (0.2 μg per mouse); ▲ IL-1β (0.02 μg per mouse); ●, control; ★, $p<0.001$; ★★, $0.02<p<0.05$

Protective activity of IFN-γ, G-CSF, GM-CSF and IL-1β on herpes simplex virus (HSV) infection in immunocompromised mice

Subcutaneous administration of MDP-Lys(L18), 1 day and 3 days before infection with HSV, almost completely protected the mice that had received intraperitoneally cyclophosphamide one day before the infection (unpublished results). To evaluate the efficacy of IFN-γ, G-CSF, GM-CSF and IL-1β against HSV infection, various doses of the cytokines were administered subcutaneously four times a day before the infection. *Table 2* and *Figure 4* show that MDP-Lys(L18) has a protective action, whereas

IFN-γ, G-CSF and IL-1β were not effective. GM-CSF showed significant protective activity (*Figure 4*).

Discussion

We have already established a model of Sendai virus infection in mice, suitable for application to human pneumonitis caused by influenza virus infection, and we have also reported that the i.n. administration of synthetic adjuvants such as MDP derivatives and chitin derivatives has been found to be a more effective route than i.v., i.p. or s.c. administration[6,7,16]. Our results clearly show that i.n. administration of IL-1β, G-CSF and GM-CSF as well as IFN-γ is an effective protection against Sendai virus infection in mice. The protection afforded by the cytokines used in this study was not attributable to contamination by endotoxins, as the endotoxins in each sample [as assayed by Pyro Dick (Seikagaku Kogyo Co. Ltd, Tokyo, Japan)] were <1 ng ml^{-1}.

The protection afforded by IFN-γ, G-CSF and IL-1β against Sendai virus infection seemed to depend on the route or the timing of administration. Although i.n. administration was effective at a dose of 10 U, intravenous administration of 10^3 U was not effective (*Figure 1*). Intranasal administration of G-CSF 1 day before infection was also effective whereas i.v. or s.c. administration were not (*Table 1*, Experiment 2). Intranasal administration of G-CSF 1 day before infection was effective (*Table 1*, Experiment 1); i.n. ad[illegible]tion of GM[illegible]s effective when it was [illegible]ministere[illegible]ither [illegible]y or 3 da[illegible] before infection (*Ta[illegible]e 1*, Experiment 3[illegible]These results suggest that i.n. adr[illegible]istration of these c[illegible]kines is likely to cause an inflamm[illegible]ory response, or th[illegible]t activates the immune system at [illegible]e administration [illegible] (lungs) and consequently stimula[illegible]s host resis[illegible]ce a[illegible]st the viral infection. Intranasal [illegible]inistr[illegible]n of M[illegible]Lys(L[illegible] was able to activate phagocytes in the lungs and the cells were able to suppress the growth of Sendai virus during the early phase of infection[17]. We observed that i.n. administration of IFN-γ was more effective than i.v. administration in activating alveolar macrophages into their cytotoxic state against tumour cells (data not shown). Matsumoto *et al.* have reported that i.p. administration of G-CSF causes a significant increase in the peritoneal exudate cells and leads to the elimination of challenged bacteria in normal or in immunocompromised mice[15]. CSF has been known to play a dual physiological role: it acts both to expand the macrophage-granulocyte population and also to enhance the functional activities of these cells[17]. Subcutaneous administration of IL-1β leads, by its chemoattractive properties, within 1 h to the migration and accumulation of phagocytes around the administration site[18]. These findings suggest that the protection against Sendai virus infection afforded by intranasal administration of IFN-γ, G-CSF, GM-CSF and IL-1β may be attributable to the activation of alveolar macrophages or neutrophils. Only IL-1β showed any therapeutic activity against Sendai virus infection when it was administered simultaneously with the infection (2 h after infection) but it was not effective if it was administered either 1 day, 3 days or 5 days after infection (*Figure 3a, b*). The Sendai virus started to grow in the lung 10 h after infection and reached its maximum titre 2 days after infection[7]. Intranasal administration of IL-1β 2 h after infection may cause rapid accumulation of phagocytes in the lungs before Sendai virus starts to grow. The precise details of the mode of action of IL-1β are now being investigated.

It has been reported that macrophages have a major role in the protection of mice against HSV infection[19–21]. Of the four cytokines used in this study, only GM-CSF showed any significant protective activity against HSV infection in the Cy-treated mice.

In the present study we have attempted to evaluate the efficacy of IFN-γ, G-CSF, GM-CSF and IL-1β as protection against Sendai virus (local infection) and HSV (systematic infection) infections in normal and immunocompromised mice, respectively. The results show that the most effective route for the administration of these four cytokines against the Sendai virus infection is the intranasal route[6,15]. Only GM-CSF protected Cy-treated mice from systematic HSV infection. These results should be of clinical value in the protection of human patients against influenza and herpes virus infections.

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